South African Journal of Botany 92 (2014) 147–150

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Cytotoxic and genotoxic effects of water extract of *Distephanus angulifolius* on *Allium cepa* Linn

J.C. Chukwujekwu, J. Van Staden *

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form 4 March 2014
Accepted 4 March 2014
Available online 31 March 2014

Edited by L Verschaeve

Keywords:
Distephanus angulifolius
Aqueous extract
Cytotoxicity
Genotoxicity
Allium cepa assay

ABSTRACT

Inspired by its ethnobotanical use, the aqueous extract of *Distephanus angulifolius* (DC.) H. Rob. & B. Kahn (Asteraceae) [synonym: *Vernonia angulifolia* DC.] was evaluated for cytotoxicity and genotoxicity using the *Allium cepa* assay. The extract was prepared with tap water as is done locally by traditional healers to use in the treatment of stomach ailments. Onion bulbs rooted in tap water for 24 h were exposed to 1.3, 2.6, 5.3, and 10.6 g/L of the extract for macroscopic and microscopic analyses, respectively. Maleic hydrazide was used as the positive control and tap water as the negative control. A statistically significant ($P < 0.05$) inhibition of root growth by the extract when compared with the negative control was observed. The calculated EC_{50} for the extract was 5.25 g/L. The mitotic index decreased with an increase in concentration of the extract. The extract induced chromosome aberrations which were statistically significant and dose-dependent compared to the negative control.
© 2014 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

The use of medicinal plants in traditional medicine for primary healthcare is an age old practice. About 80% of South African people use traditional medicines (Dold and Cocks, 2002). Approximately, 3000 species of plants are used for this purpose (Van Wyk et al., 1997). There are many reports on the biological activity of extracts from some of the plants in terms of therapeutic potential. However, information on the safety of most of the plants is scant. It is thus important that these plants are investigated for both as potential chemotherapeutic agents and for safety.

Distephanus angulifolius (DC.) H. Rob. & B. Kahn (Asteraceae) [synonym: *Vernonia angulifolia* DC.] (Robinson and Kahn, 1986) is a shrub or climber, commonly known as "Trailing Vernonia". It is known as 'impoqompoqwane' in Zulu. It is found from the Eastern Cape to Mozambique (Pedersen et al., 2009). The leaves are used to treat stomach ailments in traditional medicine (Hutchings et al., 1996; Pooley, 1998). Scientific research has confirmed its antimalarial property, the presence of sesquiterpene lactones (Bohlmann et al., 1978; Pedersen et al., 2009), and also antimicrobial, antioxidant, mutagenic and antimutagenic activities (Chukwujekwu et al., 2013). Being closely related to *Vernonia*, it is not surprising that it does contain sesquiterpene lactones. This study was conducted to evaluate the cytotoxic and genotoxic properties of water extract of *D. angulifolius* using the *Allium cepa* assay. This assay is highly effective and sensitive for the detection of mutagens (Rank and Nielsen, 1994). It is inexpensive, fast, and above all provides reliable and reproducible results.

2. Materials and methods

2.1. Plant collection

The leaves of *D. angulifolius* (Asteraceae) were collected at an altitude of 665 m in the Botanical Garden of the University of KwaZulu-Natal, Pietermaritzburg in April. It was identified by the Curator of the Garden, and a voucher specimen (Chukwujekwu # 1 NU) was deposited at the University of KwaZulu-Natal Herbarium. Plant material was dried in the oven (50 °C) overnight, milled into powdered form and kept in brown paper bags until use.

2.2. Extract preparation

The oven-dried and powdered leaves (80 g) were boiled in 1 L of tap water for 10 min and allowed to cool to room temperature (23 ± 2 °C). The extract was subsequently filtered with Whatman no. 1 filter paper, and then kept at 4 °C, as a stock solution until use.

* Corresponding author at: Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa. Tel.: +27 33 260 5130.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

http://dx.doi.org/10.1016/j.sajb.2014.03.001
0254-6299/© 2014 SAAB. Published by Elsevier B.V. All rights reserved.

2.3. A. cepa assay

Onion bulbs (*A. cepa*, L., 2n = 16) were purchased locally from a vegetable store. Before initiating the test, the outer scales of the bulbs were carefully removed without destroying the root primordia. A series of seven onion bulbs were placed in tap water for 24 h and subsequently series of five best growing bulbs were exposed to the test solutions (5, 10, 20, 40, 80 g/L) for four days in the dark and at room temperature (23 ± 2 °C). Tap water was used as the control. Test solutions were renewed every day during the course of the experiment. At the end of the exposure period (5th day), the lengths of ten roots from each bulb were measured. Using the weighted averages for each concentration and the control, the percentage root growth inhibition in relation to the control and the EC_{50} (the effective concentration where root growth amounts to 50% of the control) was calculated for the sample (Fiskesjo, 1985).

For the evaluation of possible chromosomal aberration, 1.3, 2.6, 5.3 and 10.6 g/L doses of the extract were used. Tap water was used as negative control while 10 mg/L maleic hydrazide (MH) was used as positive control. A series of seven onion bulbs were again grown on tap water in the dark at room temperature (23 ± 2 °C) for 24 h. Subsequently, series of five best growing bulbs were transferred to the chosen concentrations of the sample, positive control (MH) and negative control (tap water). They were exposed for 24 h in the dark and at room temperature (23 ± 2 °C). At the end of 24 h, root-tips (5–7 mm) from the bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v). Collected root-tips were hydrolyzed in 1 N HCl at 60 °C for 6 min after which they were washed in distilled water. A root tip was squashed on each slide, stained with 2% aceto-orcein for 10 min and covered carefully with a cover slip to avoid air bubbles. Good slides were sealed with fingernail polish. Ten slides were prepared for each concentration (two slides/bulb) and the control. Slides were randomly coded and scored blindly using an Olympus AX70 light/fluorescence microscope mounted with Nikon DS Ri1 camera. For induction of chromosomal aberration, 250 cells/slide (ie 2500 cells per concentration and control) and the mitotic index were calculated as the number of dividing cells per 2500 observed cells. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total number of cells scored at each concentration of the extract.

2.4. Statistical analysis

Data were analyzed using IBM SPSS version 19 statistical package. Analyses of variance were performed using ANOVA procedures. Duncan's multiple range test was used to determine significant differences ($P < 0.05$) between means.

3. Results and discussion

The results of the effects of the water extract of *D. angulifolius* on root growth of *A. cepa* are shown in Table 1. The cytotoxicity assay was done with five different concentrations of the extract. Besides the control, the highest root growth was recorded at 5 g/L concentration and the least growth at 80 g/L. The inhibition of root growth by the extract was dose dependent and was found to be statistically significant at $P < 0.05$ at all tested concentration when compared to the control. The EC_{50} for the extract was calculated as 5.25 g/L. Root growth inhibition in *A. cepa* is always accompanied with reduction in the number of dividing cells (Akinboro and Bakare, 2007) which takes place at the apical meristem and the appearance of stunted roots indicates the retardation of growth and cytotoxicity (Yildiz et al., 2009). *Distephanus* is closely related to *Vernonia* and they are good sources of sesquiterpene lactones. The sesquiterpene lactones vernangulide A, vernangulide B, vernodalol, vernodalin and dihydroxyvernodalin were reported in *D. angulifolius* (Pedersen et al., 2009). This class of compounds has been reported to have cytotoxic and antitumoral activities (Kupchan et al., 1969; Jasaka et al., 1992; Izevbigie, 2003). The water extracts and water soluble fractions of *Vernonia amygdalina* reduced the viability of MCF-7 and BT-549 cells respectively in a dose-dependent manner and also inhibited DNA synthesis (Gresham et al., 2008). The inhibition of root growth shown by water extract of *D. angulifolius* could be due to the presence of sesquiterpene lactones that can potentially inhibit DNA synthesis that would have otherwise promoted normal cell division.

Table 2 summarizes the cytological effects of different concentrations of water extract of *D. angulifolius* on mitotic index and chromosome behavior in the root meristematic cells of *A. cepa*. Dividing cells were observed in all the treated concentrations. The mitotic index (MI) recorded in the control was 6.16. With increasing concentrations of the extract, there was a dose-dependent decrease in the MI. It was highest at 1.3 g/L which was not statistically different ($P > 0.05$) from the negative control. The rest of the concentrations, including the positive control, yielded statistically different MI with the lowest MI with 10.6 g/L concentration. The MI which is characterized by the total number of dividing cells in the cell cycle is used as a parameter to evaluate the cytotoxicity of an agent. The level of cytotoxicity of an agent can be determined by the increase or decrease in the MI (Fernandes et al., 2007). In comparison with the negative control, a significant lower MI can indicate changes, resulting from the effect of the test agent in the growth and development of exposed organisms while a higher MI is the result of the induction of increased cell division, which may characterize an event detrimental to cells, leading to uncontrolled proliferation and even tumor formation (Hoshina, 2002). The mitodepressive effect is an indication that the extract exerted some effects on cell division of *A. cepa*. The inhibition of the cell cycle by mitodepressive agents was explained as the blockage of the G1 phase and consequently the inhibition of deoxyribonucleic acid (DNA) synthesis (El-Ghamery et al., 2000). It has also been attributed to the blockage of the G2 phase of the cell cycle and thus preventing cells from entering mitosis (Sudhakar et al., 2001), and blockage of the synthesis of nucleoproteins (Mercykutly and Stephen, 1980). According to Christopher and Kapoor (1988), the reduction of MI might be due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis.

The types and percentages of chromosomal aberrations (CAs) induced by treatments are shown in Table 2. The percentage of aberrant cells was lowest in the negative control (0.32) and it increased with an increase in the concentration of the test solution. However, at the highest concentration (10.6 g/L) tested, the percentage of aberrant cells decreased. This could be as a result of cytotoxicity of the extract

Table 1
Effects of water extract of *D. angulifolius* on root growth of *A. cepa*.

Doses (g/L)	Mean root length (mm) ± S.E.	Growth (%)	Decrease (−) in growth (%)
Control (tap water)	51.67 ± 1.01	100	–
5	31.13 ± 0.54*	60.26	39.74
10	25.47 ± 1.20*	49.29	50.71
20	15.80 ± 0.93*	30.58	69.42
40	14.67 ± 1.05*	28.39	71.61
80	12.46 ± 1.25*	24.13	75.87
EC_{50}	5.25 g/L		

* $P < 0.05$, level of significance of root growth inhibition compared with the untreated control (tap water).

Table 2
Cytogenetic analysis of *A. cepa* root-tips exposed to different concentrations of water extract of *D. angulifolius*.

Doses (g/L)	No of cells	No. of dividing cells	MI ± S.E.	Chromosome aberration					
				C-mitosis	Breaks	Bridges	Vagrant	Stickiness	% of aberrant cells
Tap water	2515	155	6.16 ± 0.45	–	–	3	3	2	0.32 ± 0.05
1.3	2518	147	5.84 ± 0.94	4	3	4	4	9	0.95 ± 0.07
2.6	2513	64	2.55 ± 0.54*	2	1	6	4	14	1.07 ± 0.28*
5.3	2465	45	1.83 ± 0.36*	4	2	7	5	10	1.14 ± 0.31*
10.6	2545	14	0.55 ± 0.42*	1	2	3	1	3	0.39 ± 0.25
MH (10 mg/L)	2509	50	1.99 ± 0.15*	2	3	5	4	9	0.92 ± 0.17*

* $P < 0.05$, level of significance of root growth inhibition compared with the untreated control (tap water).

which was reflected in the very low MI at that concentration (Table 2). There were very few dividing cells at the highest concentration of the tested sample. Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal materials. These abnormalities were observed in metaphase, anaphase and telophase stages of mitosis. The CAs observed include c-mitosis, chromosomal breaks, chromosomal bridges, vagrant and sticky chromosomes (Fig. 1). Generally, the tested extract induced more of the physiological aberrations (stickiness, c-mitosis, and vagrant chromosomes) than the clastogenic aberrations (breaks and bridges) (Table 2 and Fig. 1). Stickiness indicates a highly irreversible type of toxic effect (Fiskesjo, 1985) of the extract and it could be due to subchromatid linkage between chromosomes (Ajay and Sarbhoy, 1988; Chauhan et al., 1986; Kovalchuk et al., 1998; McGill et al., 1974). Mercykutly and Stephen (1980) are of the opinion that stickiness may be due to the depolymerization of DNA, partial dissolution of nucleoproteins, breakage and exchanges of the basic folded fiber units of chromatids and the stripping of the protein covering of DNA in chromosomes. Chromosome bridges, observed during anaphase and telophase stages (Fig. 1) were probably formed by breakage and fusion of chromosomes and chromatids, or during the translocation of the unequal chromatid exchange (Liman et al., 2010). According to El-Ghamery et al. (2000) and Luo et al. (2004), bridges cause structural chromosome mutations. C-mitosis (Fig. 1), a possibly reversible effect, might occur due to disturbed spindle apparatus and could induce aneuploidy when not reversed (Fiskesjo, 1985; Odeigah et al., 1997). The induction of vagrant chromosomes which is as a result of spindle irregularity, leads to the separation of an unequal number of chromosomes in the daughter nuclei and subsequent formation of daughter cells with unequal sized or irregularly shaped nuclei at interphase (El-Ghamery et al., 2003). Chromosome breaks, another CA observed, according to Saxena et al. (2005), indicate the clastogenic potential of a test compound.

Although previous studies (Chukwujekwu et al., 2013) indicated that the sample is not mutagenic in the Ames test without metabolic activation, it might be that, as a mixture, it contains indirect acting mutagens.

4. Conclusions

An aqueous leaf extract of *D. angulifolius* induced inhibition of *A. cepa* root growth, a mitodepressive effect on cell division and induced CAs during cell division. The types of CAs induced by the extract suggest that it could be clastogenic and also has the potential of inducing polyploidy. Considering the above results, caution should be taken

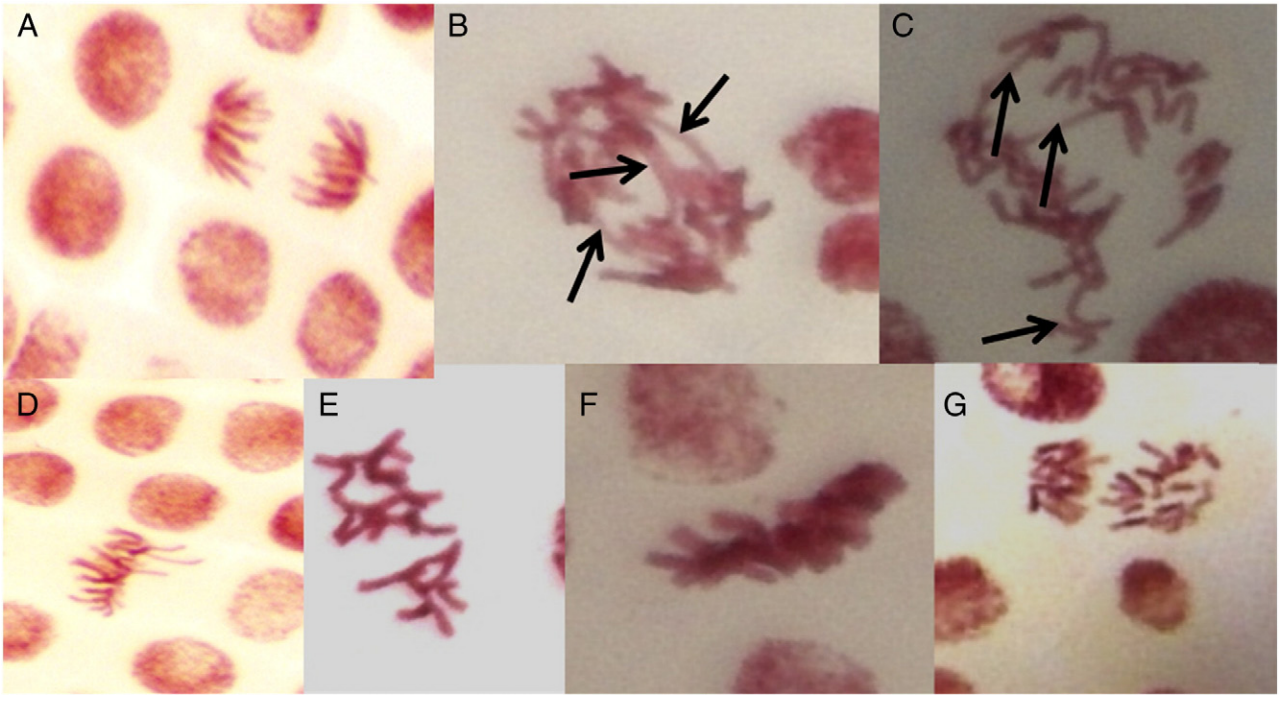

Fig. 1. *Allium cepa* meristematic cells exposed to water extract of *D. angulifolius*. (A) normal anaphase; (B) abnormal anaphase with multiple bridges; (C) abnormal anaphase with multiple bridges and chromosomal break; (D) normal metaphase; (E) c-mitosis at metaphase; (F) stickiness at metaphase; (G) abnormal anaphase with multiple breaks.

during therapeutic use of *D. angulifolius*. To reach a final conclusion on the cytotoxicity and genotoxicity potential of the extract, it has to be tested in different cytotoxicity and genotoxicity test systems, especially on animal and human cells (*in vitro* and *in vivo*).

Acknowledgements

The first author thanks the University of KwaZulu-Natal Research Office for the award of a postdoctoral fellowship.

References

Ajay, K.L., Sarbhoy, R.K., 1988. Cytogenetic studies on the effect of some chlorinated pesticides. Cytologia 53, 427–436.
Akinboro, A., Bakare, A.A., 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. Journal of Ethnopharmacology 112, 470–475.
Bohlmann, F., Brindöpke, G., Rastogi, C., 1978. A new type of germacranolide from *Vernonia* species. Phytochemistry 17, 475–482.
Chauhan, L.K.S., Dikshith, T.S.S., Sundararaman, V., 1986. Effect of deltamethrin on plant cells I. Cytological effects on the root meristem cells of *Allium cepa*. Mutation Research 171, 25–30.
Christopher, H.B., Kapoor, M.B., 1988. The cytogenetic effects of sodium salicylate on the root meristem cells of *Allium sativa* L. Cytologia 54, 203–209.
Chukwujekwu, J.C., Amoo, S.O., Van Staden, J., 2013. Antimicrobial, antioxidant, mutagenic and antimutagenic activities of *Distephanus angulifolius* and *Ormocarpum trichocarpum*. Journal of Ethnopharmacology 148, 975–979.
Dold, A.P., Cocks, M.L., 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. South African Journal of Science 98, 589–597.
El-Ghamery, A.A., El-Nahas, A.I., Mansour, M.M., 2000. The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acids content in root meristems of *Allium cepa* and *Vicia faba*. Cytologia 55, 209–215.
El-Ghamery, A.A., El-Kholy, M.A., El-Yousser, A., 2003. Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutation Research 537, 29–41.
Fernandes, T.C.C., Mazzero, D.E.C., Marin-Morales, M.A., 2007. Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pesticide Biochemistry and Physiology 88, 252–259.
Fiskesjo, G., 1985. The *Allium* test as a standard in environmental monitoring. Hereditas 102, 99–112.
Gresham, L.J., Ross, J., Izevbigie, E.B., 2008. *Vernonia amygdalina*: anticancer activity, authentication and adulteration detection. International Journal of Environmental Research and Public Health 5, 342–348.
Hoshina, M.M., 2002. Evaluation of a Possible Contamination of the Waters of the Claro River-Municipality of Rio Claro, Part of the Corumbatai River Basin, with the Mutagenicity Tests Using *Allium cepa*. State University of Sao Paulo, Rio Claro, SP.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.
Izevbigie, E.B., 2003. Discovery of water soluble anticancer agents (Edotides) from vegetables found in Benin City, Nigeria. Experimental Biology and Medicine 228, 293–298.
Jasaka, M., Ohigashi, H., Takagaki, T., Nozaki, H., Tada, T., Hiroto, M., Irie, R., Huffman, M.A., Nishida, T., Kaji, M., Koshimizu, K., 1992. Bitter steroid glucosides, Vernoniosides A1, A2, A3 and related B1 from possible medicinal plant-*Vernonia amygdalina* used by wild chimpanzees. Tetrahedron 48, 625–632.
Kovalchuk, O., Arkhipov, I., Telyuk, A., Hohn, P., Kovalchuk, L., 1998. The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident. Mutation Research 415, 47–57.
Kupchan, S.M., Hemingway, R.J., Karim, A., Werner, D., 1969. Tumor inhibitors. XLVII. Vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. Journal of Organic Chemistry 34, 3908–3911.
Liman, R., Akyıl, D., Eren, Y., Konuk, M., 2010. Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/*Salmonella* and *Allium* test. Chemosphere 80, 1056–1061.
Luo, L.Z., Werner, K.M., Gollin, S.M., Saunders, W.S., 2004. Cigarette smoke induces anaphase bridges and genomic imbalances in normal cells. Mutation Research 554, 375–385.
McGill, M., Pathak, S., Hsu, T.C., 1974. Effects of ethidium bromide on mitosis and chromosomes: a possible material basis for chromosome stickiness. Chromosoma 47, 157–167.
Mercykutly, V.C., Stephen, J., 1980. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. Cytologia 45, 769–777.
Odeigah, P.G.C., Nurudeen, O., Amund, O.O., 1997. Genotoxicity of oil field wastewater in Nigeria. Hereditas 126, 161–167.
Pedersen, M.M., Chukwujekwu, J.C., Lategan, C.A., Van Staden, J., Smith, P.J., Staerk, D., 2009. Antimalarial sesquiterpene lactones from *Distephanus angulifolius*. Phytochemistry 70, 601–607.
Pooley, E., 1998. A Field Guide to Wild Flowers: Kwazulu-Natal and the East Region. Natal Flora Publications Trust, Durban.
Rank, J., Nielsen, M.H., 1994. Evaluation of the *Allium* anaphase B telophase test in relation to genotoxicity screening of industrial wastewater. Mutation Research 312, 17–24.
Robinson, H., Kahn, B., 1986. Trinervate leaves, yellow flowers, tailed anthers, and pollen variation in *Distephanus Cassini* (Vernonieae, Asteraceae). Proceedings of the Biological Society of Washington 99, 493–501.
Saxena, P.N., Chauhan, L.K.S., Gupta, S.K., 2005. Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: spectroscopic basis of chromosome damage. Toxicology 216, 244–252.
Sudhakar, R., Ninge Gowda, K.N., Venu, G., 2001. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia 66, 235–239.
Van Wyk, B-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria.
Yildiz, M., Cigerci, I.H., Konuk, M., Fidan, A.F., Terzi, H., 2009. Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. Chemosphere 75, 934–938.